United States
Environmental Protection
Agency

Office of Research and
Development
Washington DC 20460

EPA/600/R-95/178
April 1996

ICR Microbial Laboratory Manual

ICR MICROBIAL LABORATORY MANUAL

by

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NOTICE

The ICR Microbial Laboratory Manual was prepared by the authors in response to a request from the Office of Water for support in ICR implementation. The methods and laboratory approval components contained in the manual were based upon consensus agreements reached at several workshops attended by industry, academia and U.S. EPA personnel and input from the ICR Microbiology Implementation team, which consisted of U.S. EPA personnel from the Office of Research and Development, Office of Water and representatives from Regional Offices. The manual has been peer reviewed by experts outside of U.S. EPA in accordance with the policy of the Office of Research and Development. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ACKNOWLEDGMENTS

The contributions from Robert S. Safferman, Robert H. Bordner and John A. Winter, the helpful suggestions from members of the ICR Microbiology Implementation Team, the graphical support of Fred P. Williams Jr. and the secretarial assistance of Mary Ann Schmitz and Cordelia Nowell are greatly appreciated.

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SECTION I. INTRODUCTION

BACKGROUND ON THE INFORMATION COLLECTION RULE (ICR)

The United States Environmental Protection Agency (U.S. EPA) instituted a formal regulation negotiation process in 1992 to develop the Disinfectant/Disinfection By-Product (D/DBP) Rule.[1] The Advisory Committee that was established to negotiate the regulation included representatives from the water industry, State health agencies, environmental groups, consumer groups, and the U.S. EPA. During negotiations, the Advisory Committee realized that setting strict limits on the levels of disinfectants and disinfection by-products (D/DBPs) in drinking water could result in increasing risk of waterborne disease from pathogens. To balance the risks from pathogens and chemicals, the Advisory Committee made several recommendations and the final result was the development of three new drinking water regulations.

The Disinfectant/Disinfection By-Product Rule was the primary rule negotiated. The Advisory Committee recommended a two step approach to regulating the D/DBPs with the first stage of the regulation coinciding with a regulation to ensure microbial safety of the water. The Stage 1 D/DBP Rule: 1) sets limits on the amount of disinfectants allowed in drinking water; 2) reduces the limits on total trihalomethanes (TTHMs) from 100 μg/L to 80 μg/L; 3) sets limits on additional DBPs (sum of five haloacetic acids [HAA5], chlorite, and bromate); 4) requires the use of enhanced coagulation by utilities treating surface water containing total organic carbon (TOC) concentrations above certain levels; and 5) applies to all community and non-transient noncommunity water systems.

The second rule developed during the negotiation process is the Enhanced Surface Water Treatment Rule (ESWTR). It specifies levels of treatment to control pathogens in drinking water based on microbial quality of the source water. This rule would become effective at the same time as the Stage 1 D/DBP Rule.

The third rule that was recommended by the Advisory Committee is the Information Collection Requirements Rule (ICR). This rule addresses data needs in three areas.

The most critical element of the ICR involves the collection of data on the concentrations of specific microbes. *Cryptosporidium, Giardia* and total culturable viruses are being monitored in surface waters that are used to produce drinking water and in drinking water, when high concentrations are found in surface water. In addition, data are being collected on the concentrations of indicators of human pollution in these waters. The data collected during the ICR will be used in the development of the ESWTR.

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[1] 57 FR 53866, November 13, 1992

The second element of the ICR involves the collection of treatment plant operational data and monitoring of the source water and drinking water for general water quality characteristics, DBPs, and surrogates for DBPs and DBP precursors. These data from the ICR will be used to: 1) characterize the source water parameters that influence DBP formation; 2) determine concentrations of DBPs in drinking water; 3) refine models for predicting DBP formation; and 4) establish cost-effective monitoring techniques. Development of the Stage 2 D/DBP rule is dependent upon analyses of these data.

The third element of the ICR requires some systems to conduct bench or pilot scale studies on DBP precursor removal using either granular activated carbon or membrane filtration. The purposes of these Precursor Removal/ICR studies are: 1) to obtain more information on the cost effectiveness of these technologies for reducing DBP levels; and 2) to decrease the time systems would need to install such technology, if it was required under a Stage 2 D/DBP rule.

ENSURING DATA QUALITY FOR THE ICR

One of the major issues during development of the ICR concerned the quality of the data that would be generated during the monitoring period. The Advisory Committee recognized that the data must be both accurate and precise to meet the ICR objectives. Everyone realized the difficulty in ensuring data quality considering that the data are to be generated by many laboratories. Maintaining data comparability between laboratories would be necessary to use the data for sophisticated correlational analyses and to have data that are useful for predicting DBP formation as a function of water quality conditions. The Advisory Committee felt that the only way to ensure that useable data is obtained is for the U.S. EPA to assist the drinking water industry in identifying qualified laboratories for performing the analyses required by the ICR.

In August 1993, U.S. EPA convened a technical workgroup to assist in developing approaches for ensuring microbiological data quality. Representatives from utility, state and commercial laboratories were present at the three day meeting. Persons were invited to this meeting based on their expertise in one or more of the following areas: 1) analyzing for microorganisms; 2) day-to-day management of laboratory operations; and 3) drinking water laboratory certification programs.

The technical workgroup made several general recommendations on approaches to ensure data quality. These recommendations were included in the proposed ICR.[2] The work-group's recommendations and public comments to the proposed rule were used by the U.S. EPA to develop this manual.

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[2]59 FR 6332, February 10, 1994

SECTION II. LABORATORY QUALITY ASSURANCE PLAN

All laboratories analyzing samples for the ICR will be required to adhere to defined quality assurance procedures to ensure that generated analytical data are scientifically valid and are of known and acceptable precision and specificity. To facilitate the accomplishment of these goals, each laboratory must have a written description of its quality assurance activities, a QA plan, describing the QA management of day to day routine operations. The plan must be available for inspection for ICR laboratory approval and during the time which the laboratory is performing ICR measurements.

The laboratory's QA plan should be a separately prepared text. However, documentation for some of the listed QA plan items can be made by reference to appropriate documents, such as the laboratory's SOPs, U.S. EPA Methods, or to **Standard Methods for the Examination of Water and Wastewater**. Laboratories currently certified for coliform analyses under the Drinking Water Certification program may use their current QA Plan for ICR bacteriological monitoring. Items pertinent to the protozoan or virus analyses may be placed in an addendum. This addendum must contain all the QC criteria for these analyses.

The following items should be addressed in each QA plan:

LABORATORY ORGANIZATION AND RESPONSIBILITY

1. Include a chart showing the laboratory organization and line authority, including QA Managers.
2. List the key individuals who are responsible for ensuring the production of valid measurements and the routine assessment of QC measurements
3. Specify who is responsible for internal audits and reviews of the implementation of the QA plan and its requirements.

PERSONNEL

1. List analysts' academic background and water analysis experience.
2. Describe training available to keep personnel up to date on regulations, methods and/or TQM.

FACILITIES

Describe the following:

1. Arrangement and size of laboratories
2. Bench space
3. Storage space
4. Lighting

5. Air system
6. Lab reagent water system
7. Waste disposal system
8. Safety considerations

FIELD SAMPLING PROCEDURES

1. Identify samples collected, describe how samples are collected, sample containers, holding, transport times, and temperature.
2. Describe sample identification and information recording system, chain-of-custody procedure, if applicable.

LABORATORY SAMPLE HANDLING PROCEDURES

1. Describe sample storage conditions.
2. Describe the laboratory's sample tracking system; specify procedures used to maintain the integrity of all samples, i.e., logging, tracking samples from receipt by laboratory through analysis to disposal.

EQUIPMENT

For each equipment item describe the following:

1. Specifications
2. Calibration procedures, frequency, standards
3. Quality control records
4. Preventive maintenance and schedules, documentation

SUPPLIES

Describe the specifications for major supplies, including storage conditions for reagents and media:

1. Laboratory glassware and plasticware
2. Chemicals, reagents, dyes and culture media
3. Filters

LABORATORY PRACTICES

Describe the following practices:

1. Preparation of reagent-grade water
2. Glassware washing and preparation

3. Sterilization procedures

ANALYTICAL PROCEDURES

1. Describe all reference methods used
2. State that the analytical methods described in this manual will be followed
3. Identify available SOPs

QUALITY CONTROL (QC) CHECKS

Describe how the following are performed:

1. Confirmation/verification procedures, frequency
2. Sterility controls
3. Replicate analyses; frequency
4. QC samples, source; frequency
5. PE samples, source; frequency
6. Spiked samples
7. Between analyst deviation

DATA REDUCTION, VERIFICATION, VALIDATION AND REPORTING

Describe the procedures for the following:

1. Data reduction, e.g., conversion of raw data to mg/L., coliforms/100 mL, etc.
2. Ensuring the accuracy of data transcription and calculations.
3. Validation, e.g., how are ICR QC requirements met?
4. Reporting, including procedures and format for reporting data to utilities/EPA

CORRECTIVE ACTION CONTINGENCIES

1. Define the laboratory response to obtaining unacceptable results from PE or QC samples and from internal QC checks
2. Identify persons with responsibility to take corrective action
3. Describe how the actions taken and the effectiveness of the actions taken will be documented

RECORD KEEPING

1. Describe how records are to be maintained (e.g., electronically, hard copy, etc.)
2. Describe how long records are to be kept.
3. State where records are to be stored.

A laboratory QA plan should be concise but responsive to the above-listed items. Minimizing paperwork while improving dependability and quality of data are the intended goals. **"Preparation Aids for the Development of Category I Quality Assurance Plans,"** EPA/600/8-91/003, is a document laboratories may find useful in preparing a QA plan for the ICR. It can be obtained by calling the National Risk Management Research Laboratory, Technical Information Branch at 513-569-7562. Not all of the above sections are described in the project plan guidance (i.e., laboratory sample handling and record keeping) and the goals of a lab QA Plan in general are different from the goals of a QAPP. The former describes QA Management of day to day routine operations and the latter describes goals, interactions and procedures for a specific project. By adding to the QA lab plan what will be done to meet ICR criteria, the lab will develop a Project Plan for the ICR responsibilities.

SECTION III. LABORATORY APPROVAL PROCESS

CERTIFICATION AND LABORATORY APPROVAL PROGRAMS

Laboratories requesting approval to perform quantitative analyses for total coliforms and fecal coliforms or *E. coli* in source and drinking water for the ICR must be certified under the drinking water laboratory certification program as specified by **40 CFR 142.10(b)(4)** and **141.28**. In this program, the U.S. EPA certifies the principal State laboratory and with certain exceptions other laboratories in non-primacy states (**40 CFR 142.10**). Each State certifies all other drinking water laboratories within the State.

Laboratories that will perform quantitative analyses for total coliforms and fecal coliforms or *E. coli* in source water and drinking water must provide validation of certification under the drinking water laboratory certification program. These laboratories are requested to complete the Verification of State Certification form (**Appendix A**) and return the form to the U.S. EPA Office listed.

The drinking water laboratory certification program does not address analyses for the pathogens of relevance to the ICR — *Giardia, Cryptosporidium* and total culturable viruses. Rather than broaden the drinking water laboratory certification program to cover these organisms, U.S. EPA has developed a separate program for the ICR, using the term "laboratory approval" rather than "laboratory certification."

The final ICR Rule does not require that water systems monitor for *Clostridium* or coliphage and these organisms are not included in the laboratory approval program. However, water systems may monitor for them and submit their data to the ICR database. If this option is chosen, the methods and QC conditions described in this manual should be followed.

A major difference between the drinking water laboratory certification and laboratory approval programs is that the latter requires that certain laboratory personnel be individually approved. All principal analysts must be approved for protozoan analyses (*Giardia* and *Cryptosporidium*). All principal analysts and all analysts must be approved for analysis of total culturable viruses. Each approved laboratory and each individual who must be individually approved will be assigned a unique identification (ID) number or code.

DESCRIPTION OF APPROVAL PROCEDURE

Laboratory approval will require: (1) submission and acceptance of an application for approval, (2) satisfactory analyses of unknown Performance Evaluation (PE) samples; and (3) passing an on-site laboratory evaluation.

The term “analyst” will be used in the remainder of this section to refer to all principal analysts and analysts who must be individually approved. Only these analysts must obtain

successful performance with PE samples. They will also be required to demonstrate method proficiency during the on-site visit.

Application for Approval:

The laboratory approval process for pathogen testing will begin when the laboratory director makes a formal request for approval to the:

ICR Laboratory Coordinator
U.S. EPA, Office of Ground Water & Drinking Water
Technical Support Division
26 West Martin Luther King Drive
Cincinnati, OH 45268

Upon receipt of the formal request for approval, the U.S. EPA Laboratory Coordinator will provide the requesting laboratory an application form to be completed and returned. Only laboratories that meet the minimal facility, equipment and personnel requirements described in the application package will be considered for approval. The application package is reproduced as **Appendix B**.

Laboratories will be notified in writing when their application for approval is accepted. Laboratories meeting the minimal requirements will receive one copy each of the appropriate sampling and methods videos and their accompanying guides and a copy of this manual. In addition, U.S. EPA will provide buffalo green monkey kidney (BGM) cells and an MPN computer program to all laboratories meeting minimal requirements for virus analyses. All laboratories meeting minimal requirements for protozoan analyses will be supplied a spreadsheet for calculating *Giardia* cyst and *Cryptosporidium* oocyst concentrations. The supplied cell line and computer programs must be used during ICR monitoring to ensure uniform results.

Quality Control Samples:

Quality Control (QC) samples containing known *Giardia* cyst, *Cryptosporidium* oocyst and virus concentrations will be provided to analysts requesting approval. These samples, which are described in detail in **Section IV**, may be used for internal QC checks and to gain method proficiency. Successful analyses on QC samples will be required for ongoing approval during the ICR monitoring period. The data from QC samples for ongoing approval must be entered into an ICR Laboratory Quality Control System software developed to track QC data and sent monthly in electronic form to the ICR Data Center at the address given below. The package containing the diskette with QC data must be postmarked no later than the last day of each month.

U.S. EPA (ICR4600)
ICR Data Center
Room 1111 East Tower
401 M Street, SW
Washington, D.C. 20460

Performance Evaluation Samples:

Under the laboratory approval program, qualified laboratory personnel must analyze satisfactorily PE sample sets to become approved and then subsequent sets every month to maintain approval. Each analyst must process the PE samples for *Giardia*/*Cryptosporidium* or total culturable viruses as normal samples as described in the method protocols (**Section VII** and **Section VIII**, respectively). While personnel who do not have to be individually approved are not required to analyze PE samples, they must process the same portions of PE samples for analysts that they would for actual water samples to be tested. A hard copy of the PE data must be sent directly to the ICR Laboratory Coordinator at the address listed above. The hard copy of the data must not include the laboratory name, address or any other identifying information. This information should be included only on a cover letter accompanying the data.

A set of PE samples for initial approval will have seven to ten samples. For laboratories analyzing protozoan samples, PE samples will consist of 1 µm nominal porosity filters containing either a blank synthetic matrix or a mixture of *Giardia* cysts and *Cryptosporidium* oocysts in various concentrations in a synthetic matrix. For laboratories analyzing virus samples, PE samples will consist of 1MDS filters containing either a blank synthetic matrix or attenuated poliovirus type 3 in various concentrations in a synthetic matrix.

A set of PE samples for ongoing approval will have two samples. The samples types will be the same as for initial approval. Conditions for maintaining ongoing approval are described in **Section VI**.

A standard statistical method will be used to determine the mean concentrations, variation and pass/fail acceptance limits for PE samples using either data from reference laboratories or overall data for each lot from all laboratories.

If an analyst fails the initial PE sample set, the analyst will have a second chance with another sample set within three months. Analysts who fail PE samples should contact the ICR Laboratory Coordinator for suggestions on remedies for the problem, since failure to meet acceptance levels on a second PE sample set will result in the analyst being excluded from the program.

On-Site Laboratory Evaluation:

The primary purpose of the laboratory on-site evaluation is to ensure valid data. The immediate objective is to evaluate the facilities and equipment of the laboratory and the analyst's ability to adhere to the monitoring protocols. The use of uniform methodology makes it possible to compare data generated by the different laboratories. The laboratory evaluation criteria that will be used during site visits is given in **Section V**.

Special Approval Requirements:

1. Sample Collections:

Appropriate sample collection is an important part of the ICR process. Sample collectors will be provided a videotape and accompanying guide describing the specified sampling procedures by the U.S. EPA. Although sample collection will be performed by the utility, the analytical laboratory must supply the utility with properly cleaned or sterile sampling apparatus modules and assist the sample collectors by providing information and guidance on the procedures and proper use of equipment to ensure sample integrity.

2. Sample Archiving:

By applying for U.S. EPA approval for virus analyses, a laboratory agrees to prepare virus archive samples as described in the Virus Monitoring Protocol (see **Section VIII**).

Each water system will notify its contracted virus analytical laboratory when the following conditions trigger archiving requirements:

a. Virus detection in finished water: when a system learns that viruses were detected in any previous finished water sample, all subsequent source and finished water samples must be archived for the remainder of the ICR monitoring period.

b. Virus detected at a level of 10,000 MPN units per 100 L (approximately 100 infectious virus particles per liter) in source water: when a system learns that viruses were detected in any previous source water sample at this density, all subsequent source and finished water samples must be archived for the remainder of the ICR monitoring period.

Archive samples must be frozen at -70°C and shipped on dry ice to the ICR Laboratory Coordinator at the address listed above; however, the samples may be stored by the analytical laboratory at -70°C and shipped periodically to the U.S. EPA as a batch.

SECTION IV. QUALITY CONTROL

Laboratories that perform microbiological analyses for the ICR are required to use the methods contained in this manual. These methods identify methods-specific quality control (QC) procedures which must be followed to ensure accurate and precise data. In addition to methods-specific QC procedures, laboratories must practice intralaboratory QC, the day-to-day checks made on internal operations, and interlaboratory QC, the maintenance of minimal standards established among a group of participating laboratories.

The following are descriptions of ICR methods-specific, intralaboratory, and interlaboratory QC procedures.

CLOSTRIDIUM METHOD-SPECIFIC QC

Analytical laboratories are responsible for developing their own internal QC program and must run positive and negative controls as described in **Section XI** (subsection 9.4). U.S. EPA will not supply QC samples.

PROTOZOAN AND VIRUS METHODS-SPECIFIC QC

A U.S. EPA contractor will prepare and ship QC stock suspensions of *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts and both high-titered and low-titered QC samples of attenuated poliovirus type 3. Upon arrival, the protozoan QC samples must be stored at 4°C and the virus QC samples at -70°C. The protozoan QC stock suspensions and the high-titered poliovirus sample may be used to gain proficiency with the methods and for the development of the laboratory's own QC tests. Methods-specific QC procedures will require that the protozoan stock suspensions be diluted. Instructions for dilution will accompany the suspensions.

Protozoan Methods-Specific Assay Controls:

The purpose of these controls is to assure that the assay reagents for the **ICR Protozoan Method For Detecting *Giardia* Cysts and *Cryptosporidium* Oocysts In Water By A Fluorescent Antibody Procedure** (see **Section VII**) are functioning, that the assay procedures have been properly performed, and that the microscope has been adjusted and aligned properly.

A. Membrane Filter Preparation:

Use the INDIRECT FLUORESCENT ANTIBODY PROCEDURE to prepare at least one positive and one negative IFA control for *Giardia* cysts and *Cryptosporidium* oocysts each time the manifold is used.

B. Negative IFA Control for *Giardia/Cryptosporidium*:

Step 1. Add 1.0 mL 1X PBS to one well of the manifold containing a Sartorius cellulose acetate filter on top of a membrane support filter. Perform the **Indirect Fluorescent Antibody Staining** and **Filter Mounting** steps.

Step 2. Using epifluorescence, scan the negative control membrane at no less than 200X total magnification for apple-green fluorescence of *Giardia* cyst and *Cryptosporidium* oocyst shapes.

Step 3. If no apple-green fluorescing cyst or oocyst shapes are found, and if background fluorescence of the membrane is very dim or non-existent, continue with examination of the water sample slides.

If apple-green fluorescing cyst or oocyst shapes are found, discontinue examination since possible contamination of the other slides is indicated. Clean the equipment, recheck the reagents and procedure and repeat the assay using additional aliquots of the sample.

C. Positive IFA Control for *Giardia/Cryptosporidium*:

Step 1. Add 500-1000 *Giardia lamblia* cysts and 500-1000 *Cryptosporidium parvum* oocysts or the Ensys positive control antigen (as specified in the kit) to one well of the manifold containing a Sartorius cellulose acetate filter on top of a membrane support filter. Perform the **Indirect Fluorescent Antibody Staining** and **Filter Mounting** steps.

Step 2. Using epifluorescence, scan the positive control slide at no less than 200X total magnification for apple-green fluorescence of *Giardia* cyst and *Cryptosporidium* oocyst shapes. Background fluorescence of the membrane should be either very dim or non-existent. *Cryptosporidium* oocysts may or may not show evidence of oocyst wall folding, which is characterized under epifluorescence by greater concentrations of FITC along surface fold lines, depending upon the manner in which the oocysts have been treated, and the amount of turgidity they have been able to maintain.

If no apple-green fluorescing *Giardia* cyst or *Cryptosporidium* oocyst shapes are observed, then the fluorescent staining did not work or the positive control cyst preparation was faulty. Do not examine the water sample slides for *Giardia* cysts and *Cryptosporidium* oocysts. Recheck reagents and procedures to determine the problem.

Step 3. If apple-green fluorescing cyst and oocyst shapes are observed, change the microscope from epifluorescence to the 100X oil immersion Hoffman modulation® or differential interference contrast objective.

At no less than 1000X total oil immersion magnification, examine *Giardia* cyst shapes and *Cryptosporidium* oocyst shapes for internal morphology.

The *Giardia* cyst internal morphological characteristics include one to four nuclei, axonemes, and median bodies. *Giardia* cysts should be measured to the nearest 0.5 µm with a calibrated ocular micrometer. Record the length and width of the cysts and the morphological characteristics observed. Continue until at least three *Giardia* cysts have been detected and measured in this manner.

The *Cryptosporidium* oocyst internal morphological characteristics include one to four sporozoites. Examine the *Cryptosporidium* oocyst shapes for sporozoites and measure the oocyst diameter to the nearest 0.5 µm with a calibrated ocular micrometer. Record the size of the oocysts and the number, if any, of the sporozoites observed. Sometimes a single nucleus is observed per sporozoite. Continue until at least three oocysts have been detected and measured in this manner.

Virus Monitoring Protocol Assay Controls :

A. Negative Assay Control for the **Virus Monitoring Protocol for the ICR** (see **Section VIII**): Inoculate a BGM culture with 0.15 M sodium phosphate, pH 7.0-7.5, using the procedures in **Section VIII, Part III — Total Culturable Virus Assay** . Do not report data from associated water samples if positive CPE is obtained in this control. Do not process any more samples until the reason(s) for the positive result is determined.

B. Positive Assay Control for the **Virus Monitoring Protocol for the ICR** (see **Section VIII**): Inoculate a BGM culture with 0.15 M sodium phosphate, pH 7.0-7.5, containing 20 PFU of attenuated poliovirus type 3, using the procedures in **Section VIII, Part III — Total Culturable Virus Assay**. Do not report data from associated water samples if CPE is not observed in this control. Do not process any more samples until the reason(s) for the negative result is determined.

C. Negative Assay Control for the optional **Coliphage Assay** (see **Section IX**): Add 1 mL of buffered 1.5% beef extract to a 16 x 150 mm test tube. Continue with **Step 2** of the **Procedure for Somatic or Male-Specific Coliphage Assay** . Do not report data from associated water samples if plaques are observed in this control. Do not process any more samples until the reason(s) for the positive result is determined.

D. Positive Assay Control for the optional **Coliphage Assay** (see **Section IX**): Add 1 mL of the diluted somatic or male-specific positive control to another 16 x 150 mm test tube. Continue with **Step 2** of the **Procedure for Somatic or Male-Specific Coliphage Assay** . Do not report data from associated water samples if the positive control counts are more than one log below their normal average. Do not process any more samples until the reason(s) for the below normal positive result is determined.

Quality Control Samples:

A. *Giardia* cyst and *Cryptosporidium* Oocyst Quality Control Samples:

1. Negative QC Sample Preparation: This control is a check on equipment, materials, reagents and technique. It involves processing a 1 µm nominal porosity cartridge filter as if it were an unknown using the indicated procedures from the **ICR Protozoan Method for Detecting *Giardia* Cysts and *Cryptosporidium* Oocysts in Water by a Fluorescent Antibody Procedure** (see **Section VII**).

   Step 1. Process a 1 µm nominal porosity cartridge filter using the FILTER ELUTION AND CONCENTRATION, FLOTATION PURIFICATION and INDIRECT FLUORESCENT ANTIBODY procedures in **Section VII**.

   Step 2. Examine the entire concentrate for *Giardia* cysts and *Cryptosporidium* oocysts using the **Microscopic Examination** procedure of **Section VII**. If any cysts or oocysts are detected, do not process any more unknown samples until the source(s) of the contamination is located and corrected.

2. Positive QC *Giardia* and *Cryptosporidium* Sample Preparation: The purpose of this control is to assure that the laboratory can recover cysts and oocysts with the indicated procedures from the **ICR Protozoan Method for Detecting *Giardia* Cysts and *Cryptosporidium* Oocysts in Water by a Fluorescent Antibody Procedure** (see **Section VII**) when they are spiked into a sample at a known level.

   Step 1. Seed 40 L (10 gal) of reagent-grade water with 1000 *Giardia* cysts and 2000 *Cryptosporidium* oocysts. Pass the spiked water through a 1 µm nominal porosity cartridge filter using the procedures found in **Part 9 - Sampling** of **Section VII**.

   Step 2. Process the filtered water using the FILTER ELUTION AND CONCENTRATION, FLOTATION PURIFICATION and INDIRECT FLUORESCENT ANTIBODY procedures in **Part 10** of **Section VII**.

   Step 3. Examine the entire concentrate for *Giardia* cysts and *Cryptosporidium* oocysts using the **Microscopic Examination** procedure of **Part 10** of **Section VII**. It is not necessary to identify internal morphological characteristic using differential interference contrast microscopy. If cysts and oocysts are not detected, do not process any more unknown samples until the reason(s) for not recovering cysts and oocysts is determined and corrected.

B. Viral Quality Control Samples:

1. Negative QC Viral Sample Preparation: This control is a check on equipment, materials, reagents and technique. It involves processing a 1MDS filter and examining that sample as if it were an unknown using the indicated procedures from the **Virus Monitoring Protocol for the ICR** (see **Section VIII**).

   Step 1. Place a sterile 1MDS filter into a standard filter apparatus.

   Step 2. Process and analyze the 1MDS filter using the procedures in **Part 2 — Sample Processing** and **Part 3 — Total Culturable Virus Assay** of **Section VIII**. If any virus is detected, do not process any more unknown samples until the source(s) of the contamination is located and corrected.

2. Positive QC Viral Sample Preparation: The purpose of this control is to assure that the laboratory can recover virus with the indicated procedures from the **Virus Monitoring Protocol for the ICR** (see **Section VIII**) when virus is spiked into a sample at a known level.

   Step 1. Place 40.0 L of reagent grade water into a sterile polypropylene container.

   Step 2. Thaw the low-titered virus QC sample containing 1 mL with 200 PFU of virus. Add the entire contents of the vial into the reagent grade water and rinse the vial with 1 mL of the water. Mix and pump the solution through a standard apparatus containing a 1MDS filter using the procedures in **Part 1 — Sample Collection Procedure** of **Section VIII**.

   Step 3. Process and analyze the 1MDS filters containing QC stock virus using the procedures in **Part 2 — Sample Processing** and **Part 3 — Total Culturable Virus Assay** of **Section VIII**. If virus is not detected, do not process any more unknown samples until the reason(s) for not recovering virus is determined and corrected.

3. Coliphage Assay: Quality Control Samples have not been developed for the coliphage assay. Each laboratory should plan and conduct its own internal QC checks.

C. Quality Control Batch:

All protozoan and virus samples processed by an analyst within one week's span shall be considered to be a **"batch"**. A week is defined as a Sunday to Saturday time frame. Each sample result must be associated with a batch number. An appropriate positive and negative QC sample set must be processed with each batch. In the case of the protozoan analysis, the QC sample set should be processed at the beginning of the week's batch, if possible.

1. Failure to obtain both a positive value in the positive QC sample and a negative value in the negative QC sample results in failure of the whole batch. Consequently, data from that batch would be excluded from the ICR database.

2. Obtaining a positive value in the positive QC sample and a negative value in the negative QC sample results in acceptance of the data from the whole batch. Data must be reported for all of the samples in that batch.

D. Flagging of Sample Data not Meeting Other Quality Control Conditions:

Other quality control conditions are described in the protozoan and virus protocols. Failure to comply with these conditions may decrease the pathogen concentrations, giving false negative results or measured values that are lower than the actual levels in water samples. If such a sample cannot be collected again within the time requirements of the final ICR rule, it should be analyzed. The associated data should be flagged, and the reason for the flag placed in the comment field of the database. The following flag conditions have been identified:

1. Thiosulfate not added to finished waters with disinfectant.

2. pH not reduced to below 8.0 for virus samples.

3. Collected sample volume outside the recommended range.

4. Partial or complete freezing of sample.

5. Sample shipped without ice or chemical ice.

6. Sample arrived at ambient temperature (insufficiently cooled -- not cold to touch).

7. Maximum shipping holding time exceeded.

8. Maximum sample process or analysis time requirements exceeded.

9. Cytotoxicity in virus assays.

Samples that do not conform to other lab- or utility-specified QA conditions should be treated according to the appropriate QA plans.

EPA will convert flagged data from **"quantitative"** to **"qualitative"** by changing all positive values to "PD." All less than detection limit values will be changed to "ND." PD indicates that pathogens were detected under conditions where pathogen levels are likely to be higher than the value actually measured. ND indicates that pathogens were not detected either

due to their absence from samples or due to conditions that result in obtaining values that are less than the detection limit.

Several conditions, including the lack of a temporal relationship between finished and raw water samples and the possible addition of high pathogen numbers through recycling of filter backwash water, create the possibility of observing higher pathogen levels in finished water than in raw water. Due to this possibility, water systems must not flag such data, unless the conditions listed above apply.

INTRALABORATORY QC PROCEDURES

The following minimal quality control procedures should be followed for laboratory equipment, reagents and supplies. See **Section V** and **Appendix C** and **D** for detailed procedures as they relate to the protozoa and virus methods.

pH meter:

Standardize the pH meter prior to each use with pH 7.00 and pH 4.00 standard buffers for solutions with pH values less than 7.0 and pH 7.00 and pH 10.00 standard buffers for solutions with pH values greater than 7.0.

Date standard buffer solutions upon receipt and when opened. Discard before expiration date.

Balance (top loader or pan):

Calibrate balance monthly using Class S or S-1 reference weights (minimum of three traceable weights which bracket laboratory weighing needs) or weights traceable to Class S or S-1 weights. Calibrate non-reference weights annually with Class S or S-1 reference weights.

Maintain service contract or internal maintenance protocol and maintenance records. Conduct maintenance annually at a minimum.

Temperature Monitoring Device:

Check calibration of each in-use glass/mercury thermometer annually and of each in-use dial thermometer quarterly, at the temperature used, against a reference National Institute of Standards and Technology (NIST) thermometer or one that meets the requirements of NIST Monograph SP250-23.

Recalibrate continuous recording devices annually which are used to monitor incubator temperature using the NIST reference thermometer described above.

Incubator Unit:

Record temperature once per day for each workday in use.

Autoclave:

Record date, contents, sterilization time, and temperature for each cycle. Establish a service contract or internal maintenance protocol, and maintain records.

Use maximum-temperature registering thermometer, heat sensitive tape, or spore strips or ampules during each autoclave cycle and record temperature. Avoid overcrowding.

Check automatic timing mechanism with stopwatch quarterly.

Hot Air Oven:

Record date, contents, and sterilization time and temperature of each cycle.

Conductivity Meter:

Calibrate conductivity meter monthly with a 0.01 M KCl solution, or lower concentration if desired (see Method 120.1 in EPA, 1979 or Section 2510, **"Conductivity"** p 2-43, in APHA, 1995). An in-line conductivity meter does not need to be calibrated.

Refrigerator:

Record temperature at least once per day for each workday in use.

Ultraviolet Lamp (if used):

Test lamp quarterly with UV light meter and replace if it emits less than 70% of initial output or if agar spread plates containing 200 to 250 microorganisms, exposed to the UV light for two minutes, do not show a count reduction of 99%. Other methods may be used to test a lamp if they are as effective as the two suggested methods.

Glassware Washing:

Perform the Inhibitory Residue Test (APHA, 1995) on the initial use of a washing compound and whenever a different formulation of washing compound or washing procedure is used to ensure that glassware is free of toxic residues. Laboratories purchasing large quantities of washing compound may avoid assay problems by testing the compound on an annual basis.

Reagent Grade Water:

Test the quality of the reagent grade water or have it tested by a certified laboratory to assure it meets the criteria in **Table IV-1**.

TABLE IV-1. REAGENT GRADE WATER PURITY PARAMETERS

Parameter	Limits	Frequency
Conductivity	>0.5 megohms-cm resistance or <2 µmhos/cm at 25°C	Monthly
Pb, Cd, Cr, Cu, Ni, Zn	Not greater than 0.05 mg/L per containment. Collectively, no greater than 0.1 mg/L	Annually
Total Chlorine Residual[1]	Nondetectable	Monthly
Heterotrophic Plate Count[2]	<500/mL	Monthly
Bacteriological Quality of Reagent Water[3]	Ratio of growth rate of 0.8-3.0	Annually

[1]DPD method not required if source water is not chlorinated.
[2]Pour plate method.
[3]Test for bacteriological quality of reagent water (APHA, 1995; or Bordner and Winter, 1978). Control water for the test is defined as double distilled water using a glass unit.

INTERLABORATORY QC PROCEDURES

EPA has decided to use Performance Evaluation (PE) studies as EPA's check on inter-laboratory performance. PE samples will be distributed to the ICR laboratories on a monthly basis. See **Performance Evaluation Samples** in **Section III**.

RECORDING AND REPORTING QC DATA

Records of sample information, microbiological analyses, and method and intralaboratory QC test data are information that must be recorded and stored. Typically, the laboratory must forward sample analytical reports to the treatment plant and retain copies for its own records. Records of sample information, microbiological analyses, and method and intralaboratory QC must be kept by the laboratory for at least five years. Microbiological analysis records and methods QC data includes all raw data with calculations.

LITERATURE CITED

APHA. 1995. Standard Methods for the Examination of Water and Wastewater, 19th Ed., American Public Health Association, Washington, D.C., pp. 9-4 to 9-6.

Bordner, B. and J. Winter. 1978. Microbiological Methods for Monitoring the Environment. U.S. Environmental Protection Agency Publication No. EPA-600/8-78-017, Cincinnati, OH, pp. 200-203.

EPA. 1979. Methods of chemical analyses of water and wastes. U.S. Environmental Protection Agency Publication No. EPA/600/4-79-020, (revised 1983), Cincinnati, OH.

SECTION V. ON-SITE LABORATORY EVALUATION

GENERAL EVALUATION CRITERIA

ICR Laboratory Consultant:

On-site laboratory evaluations will be conducted by an ICR Laboratory Consultant, who will be a U.S. EPA employee or a contracted environmental microbiologist. The ICR Laboratory Consultant will record laboratory information during the site visit according to the evaluation criteria listed below. U.S. EPA will use the recorded information to make all decisions about laboratory approval. Contracted ICR Laboratory Consultants will not participate in approval decisions.

Evaluation Scheduling:

A U.S. EPA person or contractor designated to schedule on-site evaluations will contact the Director of the Laboratory to set a mutually agreeable date and time for the visit. The evaluation will be scheduled at a time, if possible, that will result in minimal disruption of laboratory activities; however, all personnel who will be analyzing ICR samples must be available to perform the protozoan and/or viral tests for which approval is requested. As a guide, a minimum of three days will be scheduled.

Before the on-site evaluation, the ICR Laboratory Consultant will review the information submitted on the application and, if completed, the performance of each analyst on QC and PE samples. If the laboratory has been previously evaluated in the drinking water program, the ICR Laboratory Consultant will review that report to ensure that any applicable deviations, problems, suggested changes, or improvements have been addressed and corrected.

At the start of the on-site evaluation, the ICR Laboratory Consultant will meet the Laboratory Director and all members who will perform the test procedures to discuss the general aspects of the laboratory evaluation.

The ICR Laboratory Consultant will use a checklist as a guide to ask questions and to record evaluation results. The **Checklist for Laboratory Approval for *Giardia* and *Cryptosporidium*** is given in **Appendix C**. The **Checklist for Laboratory Approval for Total Culturable Viruses** is given in **Appendix D**. The use of a checklist provides a logical sequence to ensure that all critical elements and recommended items related to the technical procedures, equipment items, chemical reagents, media requirements, and associated activities are covered. The Consultant will likely ask additional questions to ascertain the experience and knowledge of the laboratory personnel in all these areas.

Records of all method and intralaboratory QC and bench sheets must be available for inspection. Any deficiencies noted in records or bench sheets will be included in a written report.

Each person who must be individually approved (see **Section III**) will be required to demonstrate their ability to perform the analytical protozoan or virus protocol during the evaluation. The ICR Laboratory Consultant will also evaluate the ability of other personnel to perform those protocol steps for which they will be responsible during the monitoring period of the ICR. The laboratory must have sufficient reagents and materials available so that all personnel requesting analytical approval can conduct the required assays.

The ICR Laboratory Consultant may meet with the Laboratory Director and laboratory staff at the end of the on-site visit to present comments and recommendations on methodology, instrumentation, sampling, sample holding times, quality assurance, or other subjects.

A formal written report of the evaluation will be forwarded to the Laboratory Director no later than 30 days after the evaluation.

Since the ICR microanalytical program is scheduled for 18 months, only one on-site evaluation of each laboratory will be conducted.

SECTION VI. LABORATORY APPROVAL STATUS

The U.S. EPA Laboratory Approval Program is a limited coverage program established to carry out the provisions of the ICR.

APPROVAL CLASSIFICATION

Laboratories, principal analysts and analysts will be classified according to the following:

Laboratories:

Approved - the laboratory meets the requirements for physical facilities and equipment.

Not Approved - the laboratory possesses major facilities or equipment deficiencies, or does not have an approved analyst.

Only laboratories with an "**Approved**" status are qualified to analyze ICR samples. It is the water system's responsibility to ensure that only an "**Approved**" analytical laboratory is used. If the U.S. EPA notifies a system that its contracted analytical laboratory's status has been changed from "**Approved**" to "**Not Approved**," the system must take immediate steps to find another approved laboratory.

Principal Analysts/Analysts[3]:

Approved - The analyst (1) demonstrates strict adherence to the ICR analytical methods during an on-site evaluation, (2) performs satisfactorily on QC samples and (3) successfully analyzes unknown PE samples.

Conditionally Approved - The analyst does not perform satisfactorily on unknown PE samples during any six month period and has not completed analysis of the next scheduled monthly sample.

Not Approved - The analyst does not demonstrate method proficiency during an on-site evaluation, does not perform satisfactorily on QC samples or does not successfully analyze unknown PE samples.

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[3]These approval categories apply to principal analysts and analysts from laboratories performing virus analyses and to principal analysts from laboratories performing protozoan analyses.

Approval of principal analysts and analysts for the ICR is laboratory-dependent. All analysts who transfer to another laboratory lose their approval status and are not eligible to immediately perform ICR analyses at a new laboratory. The following steps must be performed before the analyst is eligible to analyze ICR samples: 1) an amended **ICR Application for Approval** (see **Appendix B**) listing the qualifications of the analyst must be submitted by the new laboratory and accepted by EPA, and 2) principal analysts and analysts (virus laboratories only) must successfully analyze an unknown PE sample set at the new facility.

CRITERIA FOR CHANGING APPROVAL STATUS

The approval status of a laboratory or analyst may be changed during the ICR according to the following criteria:

Changing Laboratory Approval Status:

It is the responsibility of analytical laboratories to notify U.S. EPA within seven days of any change (e.g., personnel, equipment, laboratory facilities, location, etc) in ICR application status. Failure to notify U.S. EPA of changes may result in loss of approval. If U.S. EPA decides that a laboratory is subject to downgrading to a "**Not Approved**" status because of the change, the Laboratory Director or owner will be notified in writing **(by registered or certified mail)** of the proposed change of classification. The Laboratory Director or owner will have seven days from the date of the notification to review the deficiency cited and respond to the U.S. EPA in writing specifying what corrective actions are being taken. The U.S. EPA will consider the adequacy of the response and notify the laboratory by mail within seven days of its approval status.

Changing Analyst Approval Status:

The approval status of analysts using the ICR analytical methods for protozoa or total culturable viruses will be changed based upon their performance on QC and PE samples (see **Figure VI-1**).

1. PE Samples: **Figure VI-2** gives the decision tree for deciding analyst approval status based upon PE sample data.

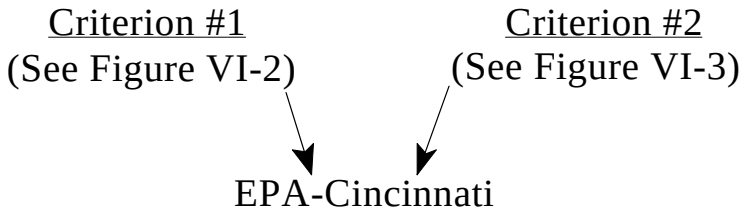
   a. An approved analyst will fail a PE sample set by submitting PE sample data that fall outside the acceptable quantitative range for the PE lots analyzed or by submitting late PE sample data. Data will be considered late if the data are not mailed to the U.S. EPA within two weeks of the shipping date of the sample to the analytical laboratory for protozoan analyzes and seven weeks for viral analyses.

FIGURE VI-1. ICR - MICROBIOLOGY DECISION TREE

There are two criteria for on-going evaluation of Approved Analysts:

- Successful Analysis of PE Samples and Timely Submission of Data (Criterion #1)
- Successful Analysis of QC Samples and Timely Submission of Data (Criterion #2)

Criterion #1 is monitored and evaluated via a contractor in Cincinnati, Ohio, using the process outlined in **Figure VI-2**. Criterion #2 is monitored and evaluated via a contractor associated with the main ICR public database (Data Central), using the process outlined in **Figure VI-3**. The recommendations of the contractors are forwarded to U.S. EPA personnel in Cincinnati for review and action.



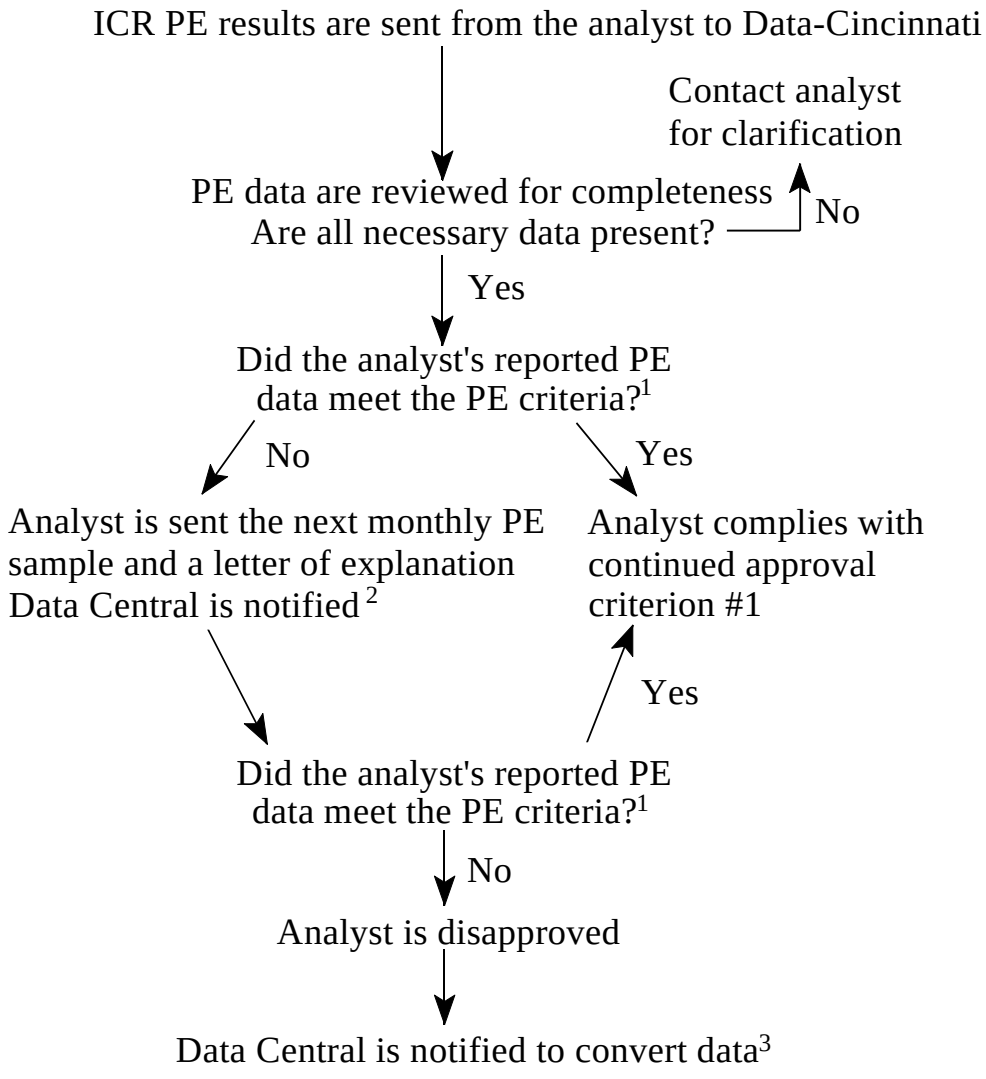
If U.S. EPA concurs with the recommendation to disapprove an analyst, the following actions will occur:

- The laboratory and the analyst will be notified of the loss of approval status for the method.
- Affected utilities will be notified.

b. Failure of an approved analyst to pass any six month set of PE samples will result in the analyst's status being changed to **"Conditionally Approved"**. The data produced by that analyst following the failure will be flagged as questionable. If the analyst passes the next PE sample set, the analyst's status will be converted to **"Approved"**, and his or her results will be accepted for ICR use.

c. If the analyst fails the next PE sample set, that analyst's status is immediately changed to "**Not Approved**." All the ICR sample data reported by that analyst from the date of analysis of the first failed PE sample to the date of the second failed PE sample will be converted from **"quantitative"** to **"qualitative"** by changing all positive values to **"PD"** and all less than detection limit values to **"ND"**. PD indicates that pathogens were detected under conditions where pathogen levels are likely to be higher than the

FIGURE VI-2. CRITERION #1 DECISION TREE



[1]Analysts do not meet PE criteria when their PE data fall outside the acceptable quantitative range for any PE sample set analyzed or if they do not report PE data to Data-Cincinnati within seven weeks for virus data and two weeks for protozoan data.

[2]Data Central will flag all data produced by an analyst since the ending date of the failed PE sample set as questionable and will convert the approval status of the analyst to "**Conditionally Approved**."

[3]Data Central will convert data received from the ending date of the 1st PE sample set to the ending date of the 2nd PE sample set from "quantitative" to "qualitative" by changing all positive values to "PD" and all less than detection limits to "NP." All data received after the ending date of the 2nd PE sample set will be deleted. The approval status of the analyst will be converted to "**Not Approved**."

value actually measured. ND indicates that pathogens were not detected either due to their absence from samples or due to conditions that result in obtaining values that are less than the detection limit. All data received after the date of analysis of the second PE sample set will be deleted.

2. QC Samples: **Figure VI-3** gives the decision tree for deciding analyst approval status based upon QC sample data.

   a. The status of an approved analyst will be changed to "**Not Approved**" if the analyst obtains invalid QC data or submits late sample or QC data for **three consecutive** batches during any sliding six month period. QC data will be considered invalid if a positive value is obtained from the negative QC sample or if the observed values obtained from positive QC samples do not fall within an acceptable range. The acceptable range will be determined by the U.S. EPA. Protozoan QC data will be considered late if results are not submitted on the first monthly QC data disk that is due two weeks after the latest sample collection date within a batch. Virus QC data will be considered late if results are not submitted on the first monthly QC data disk that is due seven weeks after the latest sample collection date within a batch. Sample data will be considered late if U.S. EPA does not receive data by the time specified in the final rule.

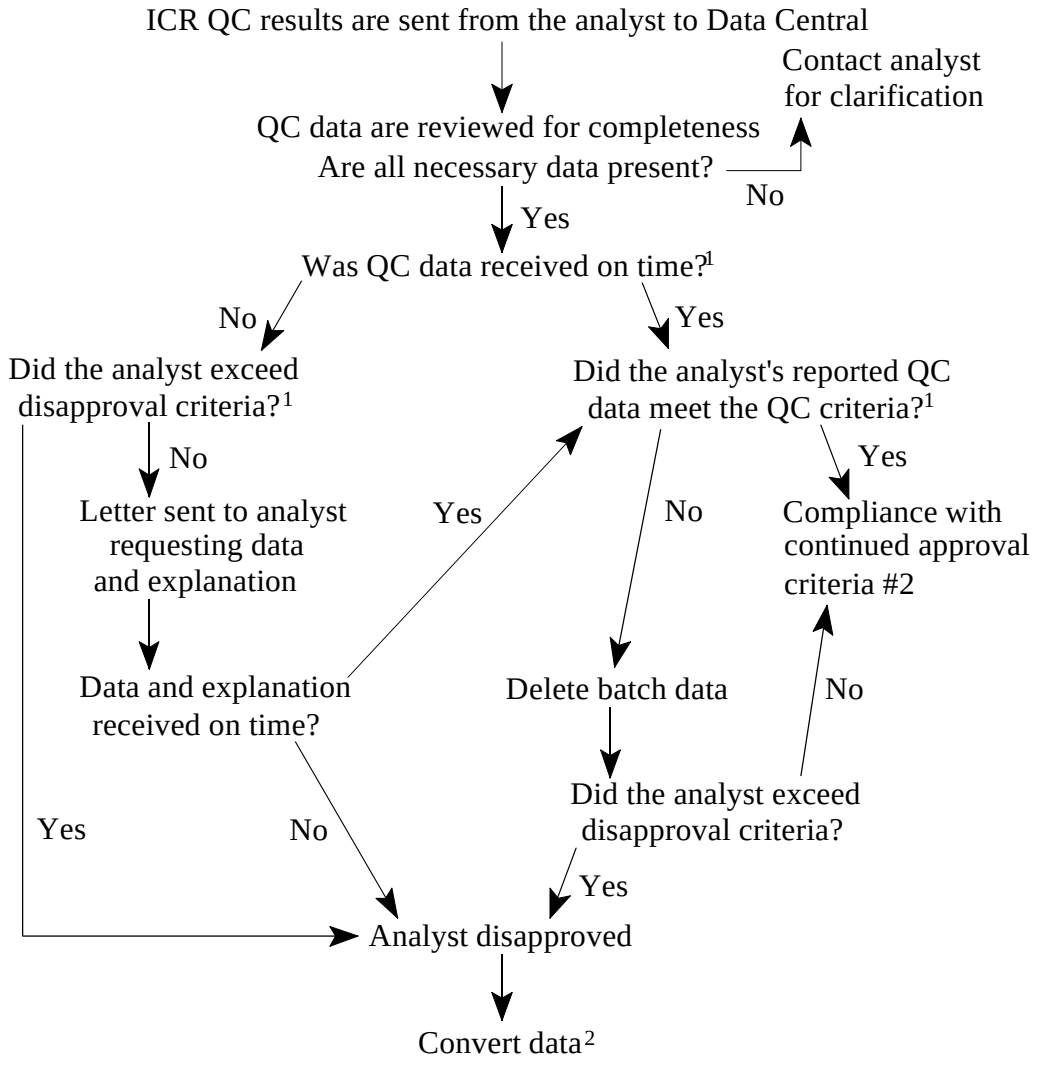
   b. The status of an approved analyst will be changed to "**Not Approved**" if the analyst obtains invalid QC data or submits late sample or QC data for **any two** batches during any sliding six month period for an analyst analyzing a batch every three to four weeks, from **any three** batches for an analyst analyzing a batch every two weeks, or from **any six** batches for an analyst analyzing a batch every week.

   c. Data received from the date of analysis of the first failed QC sample set to the date of analysis of the QC sample set leading to disapproval will be converted from **"quantitative"** to **"qualitative"** by changing all positive values to **"PD"** and all less than detection limit values to **"ND"** as above. All data received after the date of analysis of the QC sample set leading to disapproval will be deleted. The approval status of the analyst will be converted to **"Not Approved"**.

SYSTEM FOR NOTIFYING UTILITIES OF LABORATORY STATUS

EPA will maintain and make available a list of **"Approved"** laboratories. The list will be distributed directly to participating water systems, as well as each U.S. EPA Regional Office and State Primacy Agency. The list will also be available for public distribution from the U.S. EPA.

FIGURE VI-3. CRITERION #2 DECISION TREE



[1]Analysts will be disapproved for any of the following conditions: invalid QC data or late submission of data for three consecutive batches during sliding six month periods; invalid QC data or late submission of data from any two batches during sliding six month periods for analysts analyzing a batch every three to four weeks, from any three batches for analysts analyzing a batch every two weeks or from any six batches for analysts analyzing a batch every week.

[2]Data Central will convert data received from the date of analysis of the 1st failed QC sample to the date of analysis of the QC sample leading to disapproval from "quantitative" to "qualitative" by changing all positive values to "PD" and all less than detection limits to "NP." All data received after the date of analysis of the QC sample leading to disapproval will be deleted. The approval status of the analyst will be converted to "**Not Approved**."